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(54) Title: BACKBONE CYCLIZED INHIBITORS OF HEAT SHOCK PROTEINS

(57) Abstract: Novel peptide analogs which are backbone cyclized peptide antagonists of heat shock proteins are disclosed as well as methods for synthesizing them. Pharmaceutical compositions comprising backbone cyclized peptide antagonists of heat shock protein 60, and methods of using such compositions for treatment, prevention and diagnosis of inflammatory, antoimmune and infectious disorders are also disclosed.

# BACKBONE CYCLIZED INHIBITORS OF HEAT SHOCK PROTEINS

## FIELD OF THE INVENTION

The present invention relates to conformationally constrained $N^{\alpha}$ backbone-cyclized peptide analogs that are antagonists of heat shock proteins, said analogs cyclized via novel linkages, and to pharmaceutical compositions containing same. Said analogs are capable of inhibiting the binding of hsp60 to the toll-like-receptor and therefore can be used for prevention, treatment or diagnosis of inflammatory disorders and autoimmune diseases.

## BACKGROUND OF THE INVENTION

### Heat Shock Proteins

Heat shock proteins (hsp) are ubiquitous polypeptides produced by all cells of all species. Heat shock proteins are expressed both as constitutive proteins that act as molecular chaperones (Becker J. and Craig A. E., Eur. J. Biochem. 219;11, 1994), and as inducible stress proteins, and therefore are also referred to as stress proteins. The up-regulation of hsp expression within the cell was first described when cells were confronted with a sudden increase in temperature. Later, it was found that other insults also induce hsp synthesis.

Molecular chaperones are proteins that play an essential role in post-translational assembly of oligomeric proteins into complexes, intracellular folding and transport of proteins, and protection of unfolded proteins from misfolding (denaturation) or unwanted protein-protein interactions.

Heat shock proteins are among the best conserved proteins phylogenetically, with respect to both sequence and function. Human and bacterial Heat shock proteins are more than 50% homologous (Jindal et al. Mol Cell Biol. 9:2279, 1989).

Hsp60 is a mitochondrial chaperone with a major role in protein folding and unfolding as well as translocation of proteins into mitochondria. In the mitochondria it facilitates the refolding of these proteins, and, if necessary, their subsequent assembly into oligomeric complexes.

**Hsp60 in Autoimmunity and Inflammation**

Hsp60 is found in the cell cytosol under stressful and inflammatory conditions; cell infection or elevated cytokine levels will induce the cellular stress response. Therefore, it is not surprising that hsp60 is a highly immunogenic protein: it is the "common antigen" of gram-negative bacteria. Immunological reactivity to both bacterial and autologous-hsp60 is highly prevalent in the general population, since the pathogen-directed immune response can easily convert into an autoimmune response due to the high homology.

T-cell responses to multiple hsp60 epitopes are present in various autoimmune and inflammatory diseases (Van Eden et al. Immunology Today 19;303, 1998), including type 1 diabetes (Elias et al. Proc. Natl. Acad. Sci. 88:3088, 1991), rheumatoid and juvenile arthritis, multiple sclerosis, ankylosing spondylitis, pelvic inflammation-associated infertility, inflammatory bowel disease, atherosclerosis, graft rejection and more. The immune system reacts to hsp60 epitopes that are either cross-reactive between the human and bacterial analogues, or idiosyncratic.

Immunological tolerance has never been achieved so far to hsp60 epitopes in experimental systems, indicating that the immune system is predisposed to recognize this particular protein. It is a strongly immunogenic protein, in the general (healthy) population as well as in patients with inflammatory disorders.

Inflammatory diseases associated with hsp60 expression in target tissues include:

(i) Autoimmune diseases: diabetes (Birk et al. Proc Natl Acad Sci 93:1032, 1996), multiple sclerosis, rheumatoid arthritis (Van Eden et al., Nature, 331:171, 1988), juvenile chronic arthritis;

(ii) Chronic inflammation: inflammatory bowel disease, reactive arthritis;

(iii) Graft rejection (Birk et al. Proc Natl Acad Sci, 96:5159, 1999);

(iv) Atherosclerosis (Chen et al., J. Immunol. 162, 3212, 1999).

Hsp60 has been implicated in atherosclerosis, since autoantibodies to human hsp60 were demonstrated to correlate with the clinical status of patients and experimental animal models. The suggested mechanism was that autoreactive hsp60-specific T-cells respond to hsp60 that is over-expressed on endothelial cells of the aortic intima. An alternative mechanism is suggested by the finding that hsp60 is expressed in 89% of macrophages in the atheroma. Moreover, hsp60 can stimulate macrophage functions relevant to atherosclerosis, such as the production of TNFα, IL-6 and matrix-degrading metalloproteinases.

Hsp60 can contribute significantly to the inflammatory process that ends in graft rejection. By introducing hsp60-derived antagonists at an early stage after transplantation one could dampen the inflammation and prolong graft survival.

**Use of Heat shock proteins in therapy**

Hsp60 has been suggested to be involved in several autoimmune and inflammatory conditions including to juvenile rheumatoid arthritis, type 1 diabetes, multiple sclerosis, systemic lupus erythematosis, inflammatory bowel disease, bechet's disease, uveitis, thyroiditis, and atherosclerosis (Cohen I. Annu. Rev. Immunol. 9:567-89, 1991; Jaattela and Wissing Ann. Med.. 24, 249-58, 1992). In many of these diseases, Hsp60 is present in a soluble form in the circulation and anti-Hsp60 antibodies and T cells can be detected in high titer (Xu et al. Circulation. 102,14-20, 2000).

Several disclosures claim uses of heat shock proteins as immune modulators in diagnosis, treatment or prevention of autoimmune diseases. Most of the disclosures relate to bacterial heat shock protein 60 also known previously as hsp65, or fragments of these proteins.

For example, the particular protein produced by the human body during development of IDDM, which serves as a diagnostic marker for the incipient outbreak of IDDM, is the human heat shock protein having a size of about 65 KD (human hsp65) or an antigen cross-reactive therewith as disclosed in EP 0417271, and in US patents 5,114,844; 5,671,848; 5,578,303 and 5,780,034.

It has been disclosed that fragments of this hsp60 protein may serve as therapeutically useful entities in preventing or alleviating IDDM and host vs. graft disease (US patents 6,180,103 and 5,993,803 and WO 96/19236, WO 97/01959 and WO 98/08536).

In addition, fragments of hsp60 may be used as carriers for development of synthetic vaccines by increasing the immunogenicity of poorly immunogenic antigens as disclosed in US patents 5,736,146 and 5,869,058.

European patent 0262710 discloses polypeptides useful for alleviation, treatment, and diagnosis of autoimmune arthritis and similar autoimmune diseases. The claimed polypeptides are derived from bacterial protein named "Antigen A" which was identified later as mycobacterial hsp60.

WO 92/04049 discloses peptides of at least seven amino acids homologous to a fragment of Mycobacterium tuberculosis hsp60, which inhibit T-lymphocytes activation and proliferation and can protect from immune reactions and immune-related disease.

WO 89/12455 and WO 94/29459, disclose the use of stress proteins and analogs for producing or enhancing an immune response or for inducing immune tolerance, for prophylaxis or therapy of autoimmune diseases and for treating or preventing infectious or cancers. A fusion protein is claimed comprising a stress protein fused to a protein against which an immune response is desired.

WO 95/25744 discloses microbial stress protein fragments containing epitopes homologous to related mammalian epitopes – used to treat and prevent inflammatory autoimmune diseases and to prevent transplant rejection. The protective epitopes are located in short peptides comprising 5-15 amino acid sequences regions of stress proteins, that are highly conserved between microorganisms and animals.

WO 97/11966 and WO 96/10039 disclose polypeptides of up to 21 amino acids, derived from microbial heat shock protein which are useful for prophylaxis or treatment of autoimmune diseases especially arthritis.

WO 96/16083 discloses a peptide 25 amino acids long, derived from the 10 kD heat shock protein (hsp10) of Mycobacterium tuberculosis which is useful in pharmaceutical

products for the treatment of inflammatory pathologies, especially rheumatoid arthritis.

WO 91/02542 discloses the use of antigenic and/or immuno-regulatory material derived from mycobacterium vaccae and specifically hsp60, for treating chronic inflammatory disorders caused or accompanied by an abnormally high release of IL-6 and/or TNF-α.

WO 96/18646 discloses peptides of 9-20 amino acids derived from Mycobacterial hsp60 used for treatment or prevention of autoimmune CNS diseases, e.g. multiple sclerosis, chronic inflammatory CNS disease and primary brain tumors.

WO 94/02509 discloses peptides of 7-30 amino acids derived from DR3-restricted epitope of Mycobacterial hsp60 used for treatment of HLA-DR3 related autoimmune diseases.

WO 00/27870 discloses peptides derived from Mycobacterial and rat hsp60 and vaccines comprising such peptides for immunization against autoimmune and inflammatory diseases.

US 5,958,416 describes heats shock protein peptides and methods for modulating autoimmune central nervous system diseases.

Other inventors use heat shock proteins other than hsp60 for treatment. For example, US 5,348,945 discloses a method for reducing mortality in stressed tissue with heat shock protein for treatment of atherosclerosis, arterial restenosis and anoxis nerve damage using exogenous hsp70. Other inventions (e.g. JP 10212230, JP 09241159), disclose synthetic and natural compounds and extract which inhibit the expression of proteins belonging to the hsp60 or hsp27 families and are therefore useful for treating autoimmune diseases and cancers.

**Toll proteins**

The recently discovered toll receptors are speculated to represent the most ancient host defense system found in mammals, insects and plants (Kopp and Medzhitov, Curr. Opin Immunol. 11, 13-8, 1999). The family of the human receptors is described in Rock et al. (Proc. Natl. Acad. Sci. USA, 20, 588-93, 1998).

International application WO 99/20756 discloses DNA sequences encoding the human Toll proteins and antibodies specifically binding these proteins. The polypeptides are used to identify other proteins involved in Toll-mediated transduction (e.g. natural ligands), to screen for receptor and ligand mimics, and to generate antibodies.

International application WO 98/50547 discloses human DNA of Toll-like receptors and proteins and peptides derived from them. The compounds are claimed for use in altering phosphate metabolism, modulating inflammatory function or innate immune responses. A binding compound, preferably an antibody or antibody fragment which specifically binds to these proteins or peptides, is also disclosed.

The exact role of the Toll proteins in the above-mentioned processes has not been elucidated.

**Hsp60 and Toll-like-receptor 4 (Tlr4)**

The previously described ligands for Toll-like receptors in mammalian cells are of microbial origin, which is in line with a function of these receptors in innate immune responses. It was recently found that the chaperone hsp60 is a putative endogenous ligand of Toll-like receptors in mammals (Ohashi et al. J. Immunol. 164, 558-61, 2000). This finding suggests that Toll-like receptors may not only have a function in innate immune defense against microbial pathogens but serve also physiological functions by interacting with endogenous ligands.

It is noteworthy that both Toll-like receptors and hsp60 are found early in phylogeny and both are of remarkably conserved structure. This suggested that their interaction is relevant and may also occur in more primitive organisms. Mammalian hsp60 usually is sequestered to the cell interior, in accordance with its ability to function as a chaperone. However, hsp60 becomes accessible when it is set free during necrosis of tissue during inflammation or when hsp60 is partially translocated to the plasma membrane in response to diverse types of stress. It was therefore proposed that autologous hsp60 may serve as a danger signal antigen to the innate immune system (Chen et al. ibid).

The exact mechanism of interaction between mammalian hsp60 and the Tlr4

complex remains to be elucidated. Two members of the Tlr4 complex have been identified as CD14 and MD-2, both of which strongly potentiate lipopolysaccharide (LPS) responsiveness of Tlr4. For human TIr2 direct binding to LPS was demonstrated in vitro and efficient signaling appears to require serum CD14.

Thus, there is a need to modulate the role of autologous hsp60 and possibly of Toll-like receptor complexes in the regulation of pro-inflammatory immune responses and to provide molecules which are useful to suppress or even prevent such responses.

International application WO 01/43691 discloses fragments and antagonists of Hsp60 for treatment of inflammatory and autoimmune diseases. That application in its entirety is incorporated herein as a reference.

**Backbone Cyclized Peptide Analogs**

In the last few years new methods have been established for the treatment and therapy of illnesses in which peptides have been implicated. However, the use of peptides as drugs is limited by the following factors: a) their low metabolic stability towards proteolysis in the gastrointestinal tract and in serum; b) their poor absorption after oral ingestion, in particular due to their relatively high molecular mass or the lack of specific transport systems or both; c) their rapid excretion through the liver and kidneys; and d) their undesired side effects in non-target organ systems, since peptide receptors can be widely distributed in an organism.

It would be desirable to achieve peptide analogs with greater specificity thereby achieving enhanced clinical selectivity. It would be most beneficial to produce conformationally constrained peptide analogs overcoming the drawbacks of the native peptide molecules, thereby providing improved therapeutic properties.

A novel conceptual approach to the conformational constraint of peptides was introduced by Gilon, et al., (Biopolymers 31:745, 1991) who proposed backbone to backbone cyclization of peptides. The theoretical advantages of this strategy include the

ability to effect cyclization via the carbons or nitrogens of the peptide backbone without interfering with side chains that may be crucial for interaction with the specific receptor of a given peptide. Further disclosures by Gilon and coworkers (WO 95/33765, WO 97/09344, US 5,723,575, US 5,811,392, US 5,883,293 and US 6,265,375), provided methods for producing building units required in the synthesis of backbone cyclized peptide analogs. The successful use of these methods to produce backbone cyclized peptide analogs of bradykinin analogs (US 5,874,529), and backbone cyclized peptide analogs having somatostatin activity was also disclosed (WO 98/04583, WO 99/65508, US 5,770,687 and US 6,051,554). All of these methods are incorporated herein in their entirety, by reference.

None of the background art teaches or suggests the conformationally constrained backbone cyclic analogs of heat shock proteins disclosed herein.

## SUMMARY OF THE INVENTION

It is an object of the present invention to provide backbone cyclized peptides that mimic or inhibit the action of heat shock proteins or peptides derived therefrom. The analogs may be agonists or antagonists of the biological activity of the hsp molecules.

It is yet another object of the present invention to provide backbone cyclized analogs of heat shock proteins or peptides derived therefrom, having improved properties compared to the linear sequence to which they correspond. The improved properties include but are not limited to prolonged metabolic stability, or enhanced selectivity of action.

According to the present invention it is now disclosed that preferred analogs are derived from or mimic certain epitopes of the molecule designated hsp60. Most preferred peptides include analogs of the human hsp60 while additional preferred analogs include analogs of bacterial hsp60. The backbone cyclized peptide analogs may be derived from epitopes which undergo processing and are not necessarily exposed in the native protein. Conversely, the peptide analogs may be derived from epitopes or loops of the protein

which are exposed on the surface of the hsp molecule.

According to preferred embodiments of the present invention, anti-inflammatory backbone cyclized peptide analogs disclosed and claimed are characterized by their ability to act as antagonists of the inflammatory responses induced by hsp60.

It is another object of the present invention to provide novel anti-inflammatory backbone cyclized peptides that are capable of acting as antagonists of hsp60 characterized in that they have the ability to reduce or prevent the induction of a pro-inflammatory immune response of cells of the innate immune system by hsp60. Most preferred embodiments are backbone cyclized peptides, which are capable of binding the Toll-like-receptor 4 (Tlr4) thereby inhibiting the binding of hsp60 to Tlr4.

According to the present invention, novel peptide analogs of hsp60, which are characterized in that they incorporate novel building units with bridging groups attached via the alpha nitrogens of alpha amino acids, have now been generated. Specifically, these compounds are backbone cyclized analogs comprising a peptide sequence of 5 to 30 amino acids that incorporate at least one building unit, said building unit containing one nitrogen atom of the peptide backbone connected to a bridging group comprising an amide, thioether, thioester or disulfide, wherein the at least one building unit is connected via said bridging group to form a cyclic structure with a moiety selected from the group consisting of a second building unit, the side chain of an amino acid residue of the sequence or a terminal amino acid residue. Preferably, the peptide sequence incorporates 6-24 amino acids, more preferably it incorporates 7-18 amino acids.

Additional more preferred analogs according to the present invention are novel backbone cyclized peptide analogs of known hsp60 fragments which are relevant to IDDM as disclosed in US 6,180,103, WO 96/19236 and WO 97/01959.

The peptide analogs of the present invention are useful as active ingredients in pharmaceutical compositions for the prevention or treatment of diseases involving heat shock proteins in their etiology or pathology. In this respect it is an object of the present invention to provide molecules that are useful in suppression or prevention of certain diseases and conditions in which hsp60 is involved in induction of a pro-inflammatory

immune response of cells of the innate immune system such as chronic inflammatory diseases, graft rejection and autoimmune diseases. They are also useful for diagnosis or monitoring the progression of these diseases.

The present invention furthermore relates to pharmaceutical compositions comprising a backbone cyclic peptide analog according to the invention. The formulation of said compound into a pharmaceutical composition further comprises the addition of a pharmaceutically acceptable carrier, excipient and/or diluent.

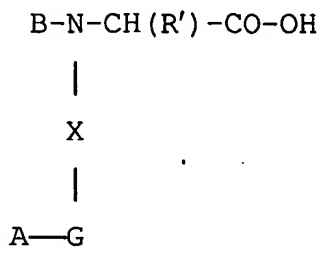
The pharmaceutical compositions according to the present invention comprise at least one backbone cyclized peptide analog of a heat shock protein. These pharmaceutical compositions may be administered by any suitable route of administration, including orally, topically, transdermally or systemically. Preferred modes of administration include but are not limited to parenteral routes such as intravenous and intramuscular injections. Additional preferred routes of administration include but are not limited to administration via nasal inhalation or oral ingestion.

Backbone cyclized analogs of the present invention are in methods for the treatment of disorders including: chronic inflammatory diseases autoimmune diseases, graft rejection, and infectious diseases including but not limited to type I diabetes, rheumatoid arthritis, reactive arthritis, juvenile chronic arthritis, multiple sclerosis, myasthenia gravis, systemic lupus erythematosus, Crohn's disease, inflammatory bowel disease, atherosclerosis, gingivitis, arterial restenosis, anoxic nerve damage, primary biliary cirrhosis, sarcoidosis, ulcerative colitis, psoriasis, Guillain-Barre syndrome, and neuro-inflammatory diseases.

Furthermore, the backbone cyclized analogs according to the present invention may be used in methods and kits for diagnosis of diseases in which Tlr4 is over-expressed and as a research tool for detection of Tlr4 by in-virto and ex-vivo detection methods. This can be facilitated by the ability of preferred compounds according to the invention to bind to the Tlr4.

These backbone cyclized peptide analogs of heat shock proteins are prepared by incorporating at least one $N^{\alpha}$-ω-functionalized derivative of an amino acids into a peptide

sequence and subsequently selectively cyclizing the functional group with one of the following: I) one of the side chains of the amino acids in the peptide sequence II) one of the termini of the peptide sequence, or III) with another ω-functionalized amino acid derivative. The $N^{\alpha}$-ω-functionalized derivative of amino acids preferably have the following formula:



Formula No. 1

wherein X is a spacer group selected from the group consisting of alkylene, substituted alkylene, arylene, cycloalkylene and substituted cycloalkylene; R' is an amino acid side chain, optionally bound with a specific protecting group; B is a protecting group selected from the group consisting of alkyloxy, substituted alkyloxy, or aryl carbonyls; and G is a functional group selected from the group consisting of amines, thiols, alcohols, carboxylic acids and esters, aldehydes, alcohols and alkyl halides; and A is a specific protecting group of G.

Preferred building units are the ω -functionalized amino acid derivatives wherein X is alkylene; G is a thiol group, an amino group or a carboxyl group; and R' is the side chain of an amino acid. Further preferred are ω-functionalized amino acid derivatives wherein R' is protected with a specific protecting group. After completion of the synthesis the protecting groups are removed.

Disclosures in the background art relate to numerous specific peptides derived from hsp60, which are useful in the treatment of specific diseases or disorders based on the interaction of those peptides with specific T-cell receptors. Are disclosures are directed to

peptides derived from hsp60 which are useful for the suppression or prevention of innate immune responses mediated by other cells of the immune system. The present application discloses novel backbone cyclized analogs of peptides derived from hsp60 and encompasses all of the known hsp60 peptides previously claimed for their capacity to modulate the T cell responses in the form of backbone cyclized analogs of the known linear sequences.

The preferred backbone cyclized analogs of the present invention are now disclosed: Currently preferred embodiments comprise backbone cyclic analog of a sequence selected from the group of:

a. Glu-Glu-Ile-Ala-Gln-Val-Ala-Thr-Ile-Ser-Ala-Asn-Gly-Asp-Lys-Glu-Ile-Gly-Asn-Ile corresponding to residues 166-185 of human hsp60;

b. Val-Leu-Gly-Gly-Gly-Cys-Ala-Leu-Leu-Arg-X2-Ile-Pro-Ala-Leu-Asp-Ser-Leu-Cys-Pro-Ala-Asn-Glu-Asp corresponding to residues 437-460 of the human hsp60, wherein Cys at positions 442 and 447 may be each substituted with Val residue and Thr at position 450 may be substituted with Lys;

c. Ile-Val-Leu-Gly-Gly-Gly-Cys-Ala-Leu-Leu-Arg-Cys-Ile-Pro-Ala-Leu-Asp-Ser-Leu-Thr corresponding to residues 436-455 of human hsp60;

d. Glu-Ile-Ile-Lys-Arg-Thr-Leu-Lys-Ile-Pro-Ala-Met-Thr-Ile-Ala-Lys-Asn-Ala-Gly-Val corresponding to residues 466-485 of human hsp60;

e. Thr-Phe-Gly-Leu-Gln-Leu-Glu-Leu-Thr-Glu-Gly-Met-Arg-Phe-Asp-Lys corresponding to residues 135-150 of Mycobacterium avium hsp65;

f. Gly-Val-Ile-Thr-Val-Glu-Glu-Ser-Asn-Thr-Phe-Gly-Leu-Gln-Leu-Glu-Leu-Thr-Glu-Gly-Met-Arg-Phe-Asp-Lys-Gly-Tyr-Ile-Ser-Gly-Tyr-Phe-Val-Thr-Asp corresponding to residues 171-240 of the bacterial Antigen A;

g. Pro-Glu-Arg-Gln-Glu-Ala-Val-Leu-Glu-Asp-Pro-Tyr-Ile-Leu-Leu-Val-Ser-Ser-Lys-Val-Ser-Thr-Val-Lys-Asp-Leu-Leu-Pro-Leu-Leu-Glu-Lys-Val-Ile-Gly corresponding to residues 207-241 of the bacterial Antigen A;

h. Thr-Phe-Gly-Leu-Gln-Leu-Glu corresponding to residues 180-186 of

Mycobacterium tuberculosis hsp65 and residues 135-141 of Mycobacterium avium hsp65, wherein any of residues Phe, Gly and Leu may be optionally substituted with another residue; Thr-AA1-AA2-AA3-Gln-Leu-Glu wherein AA1, AA2 and AA3 designate any amino acid residue;

i. Ile-Val-Gly-Leu-Thr-Leu-Glu-Asn-Ala-Asp-Leu-Ser-Leu derived from the sequence of microbial hsp60;

j. Val-Leu-Asn-Arg-Leu-Lys-Val-Gly-Leu-Gln-Val derived from the sequence of human hsp60;

k. Leu-Thr-Leu-Asn-Leu-Glu-Asp-Val-Gln-Pro-His-Asp corresponding to residues 330 to 341 of human hsp60;

l. Ala-Lys-Val-Asn-Ile-Lys-Pro-Leu-Glu-Asp-Lys-Ile-Leu-Val-Gln-Ala-Asn-Glu-Ala-Glu-Thr-Thr-Thr corresponding to residues 2 to 26 of Mycobacterium hsp10;

m. Thr-Ile-Ala-Ser-Asp-Glu-Glu-Ala-Arg-Arg-Gly-Leu corresponding to residues 3 to 14 of Mycobacterium hsp60;

n. Thr-Ile-Ala-Ser-Asp-Glu-Glu corresponding to residues 3-9 of Mycobacterium hsp60, wherein the residue Ser (at position 6) may be optionally substituted with Arg or Pro;

o. Thr-Ile-His-Tyr-Asp-Glu-Glu corresponding to residues 3-9 of Mycobacterium hsp60, wherein the residue His (at position 5) may be optionally substituted with Gln;

p. Gly-Pro-Lys-Gly-Arg-Asn-Val-Val-Leu-Glu-Lys-Lys-Trp-Gly-Ala-Pro corresponding to residues 31 to 46 of Mycobacterium tuberculosis hsp60;

q. Val-Val-Leu-Glu-Lys-Lys-Trp-Gly-Ala-Pro-Thr-Ile-Thr-Asn-Asp-Gly corresponding to residues 37 to 52 of Mycobacterium tuberculosis hsp60;

r. Thr-Val-Ile-Ile-Glu-Gln-Ser-Trp-Gly-Ser-Pro-Lys-Val-Thr-Lys-Asp-Gly-Val-Thr-Val corresponding to residues 36 to 55 of Mycobacterium tuberculosis hsp60;

s. Val-Val-Asn-Lys-Ile-Arg-Gly corresponding to residues 261-271of bacterial hsp65;

t. Leu-Lys-Pro-Gly-Leu-Glu-Lys-Asp-Phe derived from the sequence of Mycobacterial hsp60;

u. Leu-Lys-Arg-Gly-Ile-Glu-Lys-Ala-Val corresponding to residues derived from the

sequence of Mycobacterial hsp60;

v. Val-Ala-Vla-Lys-Ala-Pro-Gly-Phe-Gly-Asp-Arg-Arg-Lys-Ala-Met corresponding to residues 272-286 of Mycobacterial lepae.

Additional preferred backbone cyclized analogs are based on sequences disclosed in WO 01/43691 which are useful for the suppression or prevention of innate immune responses based on the interaction between hsp60 and Tlr4.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the finding that hsp60 is involved in several autoimmune and inflammatory conditions and that interference with its activity may be beneficial in these conditions. It was disclosed previously that fragments and peptides derived from hsp60 can act as inhibitors of hsp60, and the present invention further provides novel backbone cyclized peptide analogs of hsp60 having improved properties over linear peptides derived from hsp60.

The most striking advantages of backbone cyclization are:

1) cyclization of the peptide sequence is achieved without compromising any of the side chains of the peptide thereby decreasing the chances of sacrificing functional groups essential for biological recognition and function.

2) optimization of the peptide conformation is achieved by allowing permutation of the bridge length, direction, and bond type (e.g., amide, disulfide, thioether, thioester, etc.) and position of the bond in the ring.

3) when applied to cyclization of linear peptides of known activity, the bridge can be designed in such a way as to minimize interaction with the active region of the peptide and its cognate receptor. This decreases the chances of the cyclization arm interfering with recognition and function, and also creates a site suitable for attachment of tags such as radioactive tracers, cytotoxic drugs, light capturing substances, or any other desired label.

The backbone cyclized peptide analogs may be derived from epitopes which undergo processing and are not necessarily exposed in the native protein. Conversely, the peptide analogs may be derived from epitopes or loops of the protein which are exposed on the surface of the hsp molecule.

Chronic inflammatory processes of autoimmune or infectious nature can be dampened by backbone cyclic analogs of hsp60 antagonising the pro-inflammatory action of hsp60 on macrophages, dendritic cells, endothelial cells or other cells.

Additionally, based on the finding that hsp60 binds to the Toll-like receptor 4 complex and as a result elicits a potent pro-inflammatory response in cells of the innate immune system. Therefore, It is an object of the present invention to provide backbone cyclized peptides which are capable of binding to the Toll-receptor. Backbone cyclized analogs according to the present invention, which bind to the Toll-receptor are capable of inhibiting the binding of Hsp60 to the said receptor and thus inhibiting the inflammatory responses cascade which follows such binding.

Terminology and definitions:

The term "heat shock protein " relates to any member of heat shock proteins family also known as chaperones. The term "heat shock protein" also referred to "stress protein" a term that was used in the past to such molecules.

For the purpose of the present specification and claims, the term "hsp60" is intended to comprehend not only the 60 kD heat shock protein, but also any other related molecule which cross-reacts with polyclonal antibodies raised against a 60 kD heat shock protein of any species. This definition is specifically intended to include, although it is not limited to, the 65 kD, 30 kD, 25 kD and 47 kD proteins which have already been discovered. The hsp60 molecule was formerly designated hsp65, but is now designated hsp60 in view of more accurate molecular weight information; by either designation, the protein is the same.

The term "toll-like-receptor" refers to a member of the protein family described of receptor complexes that mediate immune responses of the innate immune system, as

disclosed for example in WO 98/50547 and WO 99/20756.

The term "antagonist of heat shock protein" in the context of the present invention means that these molecules are able to inhibit, reduce or prevent at least one of the biological actions of at least one of the heat shock protein, in the context of an immunological response and/or an inflammatory condition and/or an infectious disease and/or graft rejection.

As used herein "peptide" indicates a sequence of amino acids linked by peptide bonds. The peptides according to the present invention comprise a sequence of 5 to 30 amino acid residues, preferably 6 to 24 residues, more preferably 7 to 18 amino acids. A peptide analog according to the present invention may optionally comprises at least one bond which is an amide-replacement bond such as urea bond, carbamate bond, sulfonamide bond, hydrazine bond, or any other covalent bond.

Whenever "peptide of the invention" or "analogs of the invention" are mentioned in the present specification and claims, also salts and functional derivatives thereof are contemplated, as long as the biological activity of the peptide is maintained.

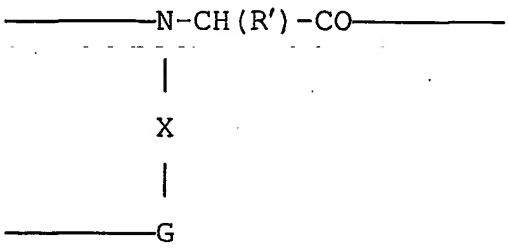
"Functional derivatives" of the peptides of the invention as used herein covers derivatives which may be prepared from the functional groups which occur as side chains on the residues or the N- or C-terminal groups, by means known in the art, and are included in the invention as long as they remain pharmaceutically acceptable, i.e., they do not destroy the activity of the peptide, do not confer toxic properties on compositions containing it and do not adversely affect the antigenic properties thereof.

These derivatives may, for example, include aliphatic esters of the carboxyl groups, amides of the carboxyl groups produced by reaction with ammonia or with primary or secondary amines, N-acyl derivatives of free amino groups of the amino acid residues formed by reaction with acyl moieties (e.g., alkanoyl or carbocyclic aroyl groups) or O-acyl derivatives of free hydroxyl group (for example that of seryl or threonyl residues) formed by reaction with acyl moieties.

"Salts" of the peptides of the invention contemplated by the invention are physiologically acceptable organic and inorganic salts.

As used herein the term "backbone cyclic peptide" or "backbone cyclic analog" denotes an analog of a linear peptide which comprising a peptide sequence of preferably 5 to 30 amino acids that incorporates at least one building unit, said building unit containing one nitrogen atom of the peptide backbone connected to a bridging group comprising an amide, thioether, thioester or disulfide, wherein the at least one building unit is connected via said bridging group to form a cyclic structure with a moiety selected from the group consisting of a second building unit, the side chain of an amino acid residue of the sequence or a terminal amino acid residue. More preferably, the peptide sequence incorporates 6-24 amino acids, still more preferably it incorporates 7-18 amino acids.

A "building unit" indicates an $N^{\alpha}$ derivatized α amino acid of the general Formula No. 2:




Formula No. 2

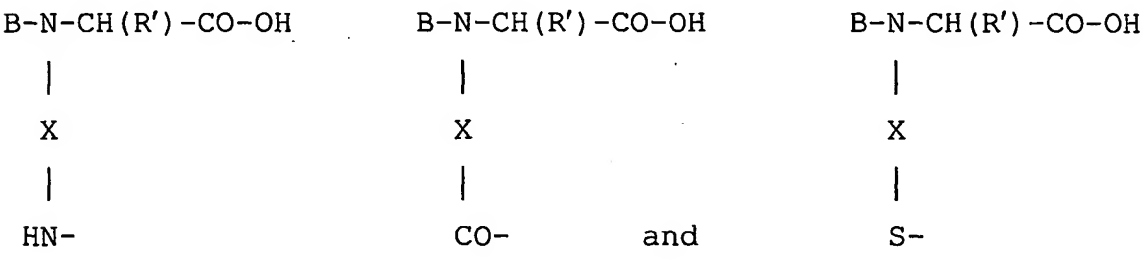
wherein X is a spacer group selected from the group consisting of alkylene, substituted alkylene, arylene, cycloalkylene and substituted cycloalkylene; R' is an amino acid side chain, optionally bound with a specific protecting group; and G is a functional group selected from the group consisting of amines, thiols, alcohols, carboxylic acids and esters, and alkyl halides; which is incorporated into the peptide sequence and subsequently selectively cyclized via the functional group G with one of the side chains of the amino acids in said peptide sequence or with another ω-functionalized amino acid derivative. The methodology for producing the building units is described in international patent applications published as WO 95/33765 and WO 98/04583 and in US 5,770,687 and 5,883,293 all of which are expressly incorporated herein by reference thereto as if set forth

herein in their entirety.

The building units are abbreviated by the three letter code of the corresponding modified amino acid followed by the type of reactive group (N for amine, C for carboxyl), and an indication of the number of spacing methylene groups. For example, Gly-C2 describes a modified Gly residue with a carboxyl reactive group and a two carbon methylene spacer, and Phe-N3 designates a modified phenylalanine group with an amino reactive group and a three carbon methylene spacer.

In generic formulae the building units are abbreviated as R with a superscript corresponding to the position in the sequence preceded by the letter N, as an indication that the backbone nitrogen at that position is the attachment point of the bridging group specified in said formulae.

The building units are ω-functionalized amino acid derivatives wherein G is an amino group, a carboxyl group, or a thiol group of the following formulae:




Formula No. 3 Formula No. 4 Formula No. 5

wherein X, R' and B are as defined above.

The compounds herein disclosed may have asymmetric centers. All chiral, diastereomeric, and racemic forms are included in the present invention. Many geometric isomers of double bonds and the like can also be present in the compounds disclosed herein, and all such stable isomers are contemplated in the present invention.
By "stable compound" or "stable structure" is meant herein a compound that is sufficiently

robust to survive isolation to a useful degree of purity from a reaction mixture, and formulation into an efficacious therapeutic agent.

The term, "substituted" as used herein and in the claims, means that any one or more hydrogen atoms on the designated atom is replaced with a selection from the indicated group, provided that the designated atom's normal valency is not exceeded, and that the substitution results in a stable compound.

When any variable (for example R, X, Z, etc.) occurs more than one time in any constituent or in any Formula herein, its definition on each occurrence is independent of its definition at every other occurrence. Also, combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

As used herein and in the claims, the phrase "therapeutically effective amount" means that amount of novel backbone cyclized peptide analog or composition comprising same to administer to a host to achieve the desired results for the indications disclosed herein.

Certain abbreviations are used herein to describe this invention and the manner of making and using it.

For instance, 2Abu refers to 2-aminobutyric acid, Alloc refer to allyloxycarbonyl, Boc refers to the t-butyloxycarbonyl radical, BOP refers to benzotriazol-l-yloxy-tris-(dimethylamino)phosphonium hexafluorophosphate, DIEA refers to diisopropyl-ethyl amine, EDT refers to ethanedithiol, Fmoc refers to the fluorenylmethoxycarbonyl radical, HBTU refers to 1-hydroxybenztriazolyltetramethyl-uronium hexafluorophosphate, HOBT refers to 1-hydroxybenzotriazole, HPLC refers to high pressure liquid chromatography, hsp refers to heat shock protein, IDDM refers to Insulin-dependent Diabetes Mellitus, kD refers to Kilo Dalton, MPS refers to Multiple parallel synthesis, MS refers to mass spectrometry, NMM refers to N-methylmorpholine, NMP refers to 1-methyl-2-pyrolidonone, PyBOP refers to Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate, PyBrOP refers to Bromo-tris- pyrrolidino-phosphonium hexafluorophosphate, and TFA refers to trifluoroacetic acid, Tlr refers to toll-like-receptor.

The amino acids used in this invention are those which are available commercially or

are available by routine synthetic methods. Certain residues may require special methods for incorporation into the peptide, and either sequential, divergent and convergent synthetic approaches to the peptide sequence are useful in this invention. Natural coded amino acids and their derivatives are represented by three-letter codes according to IUPAC conventions. When there is no indication, the L isomer was used. The D isomers are indicated by "D" before the residue abbreviation.

List of non-coded amino acids: 2Abu refers to 2-aminobutyric acid, Bpa refers to 4-Benzoylphenylalanine, Bip refers to Beta-(4-biphenyl)-alanine, Cit refers to Citruline, Dapa refers to Diaminopropionic acid, Dim refers to Dimethoxyphenylalanine, Dpr refers to Diaminopropionic acid, GlyNH2 refers to Aminoglycine, HPhe refers to Homophenylalanine, Hyp refers to Hydroxyproline, Nle refers to Norleucine, Nva refers to Norvaline, Orn refers to ornithine, Phe4C refers to 4 carboxy Phenylalanine, PheCl refers to para chloro Phenylalanine, PheF refers to para fluoro Phenylalanine, PheMe refers to para methyl Phenylalanine, Phe4$NH_2$ refers to 4 amino Phenylalanine, PheNt refers to 4 nitro Phenylalanine, Phg refers to Phenylglycine, Nva refers to Norvaline, Thi refers to Thienylalanine.

Conservative substitution of amino acids as known to those skilled in the art are within the scope of the present invention. Conservative amino acid substitutions includes replacement of one amino acid with another having the same type of functional group or side chain e.g. aliphatic, aromatic, positively charged, negatively charged. These substitutions also include replacement of Phe residues with N-Methyl-Phe residues for increasing the bio-availability of the compound and conjugation of mono- and di-saccharides moieties at the amino terminus for increasing oral bio-availability (Nelson-Piercy et al. J. Clin. Endocrinol. And Metab. 78:329, 1994), or other such substitutions as may enhance oral bioavailability, penetration into the central nervous system, targeting to specific cell populations and the like.

According to the present invention peptide analogs are cyclized via bridging groups attached to the alpha nitrogens of amino acids that permit novel non-peptidic linkages. In general, the procedures utilized to construct such peptide analogs from their building units

rely on the known principles of peptide synthesis; most conveniently, the procedures can be performed according to the known principles of solid phase peptide synthesis.

The methods for design and synthesis of backbone cyclized analogs according to the present invention are disclosed in US patents 5,811,392; 5,874,529; 5,883,293; 6,117,974, 6,265,375 and international applications WO 95/33765; WO 97/09344; WO 99/31121; WO 00/02898; and WO 00/65467. All of these methods are incorporated herein in their entirety, by reference.

The preferred backbone cyclized analogs of the present invention are now disclosed:

Currently preferred embodiments comprise backbone cyclic analog of a sequence selected from the group of:

a. Glu-Glu-Ile-Ala-Gln-Val-Ala-Thr-Ile-Ser-Ala-Asn-Gly-Asp-Lys-Glu-Ile-Gly-Asn-Ile corresponding to residues 166-185 of human hsp60;

b. Val-Leu-Gly-Gly-Gly-Cys-Ala-Leu-Leu-Arg-X2-Ile-Pro-Ala-Leu-Asp-Ser-Leu-Cys-Pro-Ala-Asn-Glu-Asp corresponding to residues 437-460 of the human hsp60, wherein Cys at positions 442 and 447 may be each substituted with Val residue and Thr at position 450 may be substituted with Lys;

c. Ile-Val-Leu-Gly-Gly-Gly-Cys-Ala-Leu-Leu-Arg-Cys-Ile-Pro-Ala-Leu-Asp-Ser-Leu-Thr corresponding to residues 436-455 of human hsp60;

d. Glu-Ile-Ile-Lys-Arg-Thr-Leu-Lys-Ile-Pro-Ala-Met-Thr-Ile-Ala-Lys-Asn-Ala-Gly-Val corresponding to residues 466-485 of human hsp60;

e. Thr-Phe-Gly-Leu-Gln-Leu-Glu-Leu-Thr-Glu-Gly-Met-Arg-Phe-Asp-Lys corresponding to residues 135-150 of Mycobacterium avium hsp65;

f. Gly-Val-Ile-Thr-Val-Glu-Glu-Ser-Asn-Thr-Phe-Gly-Leu-Gln-Leu-Glu-Leu-Thr-Glu-Gly-Met-Arg-Phe-Asp-Lys-Gly-Tyr-Ile-Ser-Gly-Tyr-Phe-Val-Thr-Asp corresponding to residues 171-240 of the bacterial Antigen A;

g. Pro-Glu-Arg-Gln-Glu-Ala-Val-Leu-Glu-Asp-Pro-Tyr-Ile-Leu-Leu-Val-Ser-Ser-Lys-Val-Ser-Thr-Val-Lys-Asp-Leu-Leu-Pro-Leu-Leu-Glu-Lys-Val-Ile-Gly corresponding to residues 207-241 of the bacterial Antigen A;

h. Thr-Phe-Gly-Leu-Gln-Leu-Glu corresponding to residues 180-186 of Mycobacterium tuberculosis hsp65 and residues 135-141 of Mycobacterium avium hsp65, wherein any of residues Phe, Gly and Leu may be optionally substituted with another residue; Thr-AA1-AA2-AA3-Gln-Leu-Glu wherein AA1, AA2 and AA3 designate any amino acid residue;

i. Ile-Val-Gly-Leu-Thr-Leu-Glu-Asn-Ala-Asp-Leu-Ser-Leu derived from the sequence of microbial hsp60;

j. Val-Leu-Asn-Arg-Leu-Lys-Val-Gly-Leu-Gln-Val derived from the sequence of human hsp60;

k. Leu-Thr-Leu-Asn-Leu-Glu-Asp-Val-Gln-Pro-His-Asp corresponding to residues 330 to 341 of human hsp60;

l. Ala-Lys-Val-Asn-Ile-Lys-Pro-Leu-Glu-Asp-Lys-Ile-Leu-Val-Gln-Ala-Asn-Glu-Ala-Glu-Thr-Thr-Thr corresponding to residues 2 to 26 of Mycobacterium hsp10;

m. Thr-Ile-Ala-Ser-Asp-Glu-Glu-Ala-Arg-Arg-Gly-Leu corresponding to residues 3 to 14 of Mycobacterium hsp60;

n. Thr-Ile-Ala-Ser-Asp-Glu-Glu corresponding to residues 3-9 of Mycobacterium hsp60, wherein the residue Ser (at position 6) may be optionally substituted with Arg or Pro;

o. Thr-Ile-His-Tyr-Asp-Glu-Glu corresponding to residues 3-9 of Mycobacterium hsp60, wherein the residue His (at position 5) may be optionally substituted with Gln;

p. Gly-Pro-Lys-Gly-Arg-Asn-Val-Val-Leu-Glu-Lys-Lys-Trp-Gly-Ala-Pro corresponding to residues 31 to 46 of Mycobacterium tuberculosis hsp60;

q. Val-Val-Leu-Glu-Lys-Lys-Trp-Gly-Ala-Pro-Thr-Ile-Thr-Asn-Asp-Gly corresponding to residues 37 to 52 of Mycobacterium tuberculosis hsp60;

r. Thr-Val-Ile-Ile-Glu-Gln-Ser-Trp-Gly-Ser-Pro-Lys-Val-Thr-Lys-Asp-Gly-Val-Thr-Val corresponding to residues 36 to 55 of Mycobacterium tuberculosis hsp60;

s. Val-Val-Asn-Lys-Ile-Arg-Gly corresponding to residues 261-271of bacterial hsp65;

t. Leu-Lys-Pro-Gly-Leu-Glu-Lys-Asp-Phe derived from the sequence of Mycobacterial hsp60;

u. Leu-Lys-Arg-Gly-Ile-Glu-Lys-Ala-Val corresponding to residues derived from the sequence of Mycobacterial hsp60;

v. Val-Ala-Vla-Lys-Ala-Pro-Gly-Phe-Gly-Asp-Arg-Arg-Lys-Ala-Met corresponding to residues 272-286 of Mycobacterial lepae;

Additional preferred backbone cyclized analogs are based on sequences disclosed in WO 01/43691 which are useful for the suppression or prevention of innate immune responses based on the interaction between hsp60 and Tlr4.

**General Methods**

General method for synthesis, purification and characterization of peptides:

The following method describes a non-limitative lab example used for production of backbone cyclized peptides according to the present invention.

Synthesis:

Resin: 1g Rink amide or Tenta-gel resin, with loading of 0.2-0.7 mmol/gr.

Fmoc- deprotection: With 7 mL of 20% piperidine in NMP. Twice for 15 minutes following 5 washes with 10 ml NMP for 2 minutes with shaking.

Couplings:

1. Regular couplings (coupling to simple amino acids): with a solution containing 3 equivalents amino acid, 3 equivalents PyBroP and 6 equivalents of DIEA in 7ml NMP. For 0.5-2 hours with shaking. Coupling is monitored by ninhydrine test and repeated until the ninhydrine solution become yellow.

2. Coupling of His and Asn with a solution containing 5 equivalents DIC and 5 equivalents HOBT in 10 ml dimethyl formamide.

3. Coupling to Gly building units: with a solution containing 3 equivalents amino acid, 3 equivalents PyBroP and 6 equivalents DIEA in 7ml NMP. Twice for 1-4 hours with shaking.

4. Coupling to building units which are not Gly: with a solution containing 5 equivalents amino acid, 1.5 equivalents triphosgen and 13 equivalents collidine in 15ml dioxane or

THF. Three times for 40 minutes at $60^0$C with shaking.

Removal of the Allyl and Alloc protecting groups of the building units: with 1.5 equivalents per peptide of Pd(PPh3)4 in 30 ml dichloromethane containing 5% acetic acid and 2.5% NMM. For 1-4 hours with shaking.

Cyclization: with a solution containing 3 equivalents PyBOP and 6 equivalents DIEA in 7ml NMP. For 0.5-2 hours with shaking. Cyclization is monitored by ninhydrine test and repeated if necessary.

Cleavage: with 82%-95% TFA supplemented with scavengers: 1-15% $H_2O$, 1-5% TIS and 1-5% EDT.

Purification:

An individual purification method for each backbone cyclic peptide is developed on analytical HPLC to give the maximum isolation of the cyclic peptide from other crude components. The analytical method is usually performed using a C-18 Vydac column 250X4.6mm as the stationary phase and water/ACN containing 0.1%TFA mixture gradient.

The preparative method is designed by implying the analytical separation method on the 2" C-18 Vydac preparative method. During the purification process, the peak containing the cyclic peptide is collected using a semi-automated fraction collector. The collected fractions are injected to the analytical HPLC for purity check. The pure fractions are combined and lyophilized.

Characterization:

The combined pure lyophilized material is analyzed for purity by HPLC, MS and capillary electrophoresis and by amino acid analysis for peptide content and amino acid ratio determination.

General method for synthesis, purification and characterization of libraries in Multiple Parallel Synthesis (MPS) format:

The MPS procedure is used as the routine peptide development procedure. Individual peptides, or groups of a few peptides, are synthesized in 96-wells microtiter plates

equipped with filters that allow passage of solvent but not of solid phase matrix. A simple and efficient valve apparatus that enable simultaneous closing and opening of all the valves (produced by Millipore) is used. The system utilizes an approach in which each well is equipped with a solvent permeable membrane at the bottom that does not pass particles above a certain size. The process allows one to place resin in the wells, perform reaction in solvent, and remove the solvent from all the wells simultaneously by applying vacuum. These special plates, which are available in the standard 96 well format allow the parallel synthesis of 96 peptides simultaneously. The synthetic scale of the procedure is in the range of 1-5 μmole per well. Following purification by C18 reverse phase columns (SepPak purification), which is also carried out in the standard 96 well format, the peptides are routinely dissolved in 1 ml of water to yield a theoretical crude concentration of 1-5 mM (depending of synthesis scale). Monitoring of chemical quality of the resulting peptides is performed by ESI-MS analysis. Analysis of several plates prepared on different occasions by different operators indicated a general success rate of about 80% as judged by the presence of the desired peptide mass in the crude preparation. Further analysis of a peptides from MPS is carried out by LC-MS. The analysis revealed crude peptide quality similar to crude preparations of peptides synthesized individually in large scale. Individual steps or the complete process is now performed automatically using automatic peptide synthesizers. According to the present exemplifications, the peptides are currently synthesized automatically using the ACT 396 of Advanced ChemTech, and the heating device Lab Tech 4 of Advanced ChemTech.

Detailed procedure for synthesis in MPS format:

For capacity of 6 μmole 10 mg resin with a substitution of 0.6 mmol/gr is used.

Fmoc deprotection: To each well 500 μl of 5% piperidine in NMP are added twice. The reaction shacked for 15 min. The NMP is removed by suction.

Washing after Fmoc deprotection: the resin is washed by placing 600 μl NMP into each well followed by evacuation of the solution by steam of nitrogen. The washing process is repeated 4 times.

Coupling using HBTU:

Well capacity: 6 μmol

Amount of amino acid per coupling per well: 30 μmol

Amino acid in NMP concentration: 0.2 M

Amino acid volume used: 150 μl

HBTU amount: 30 μmol

HBTU concentration: 0.2 M

HBTU volume used: 150 μl

DIEA added: 150 μl of 0.4 M in NMP

Total reaction volume: 450 μl

The amino acids are dissolved in a solution of HOBT in NMP. The resin is washed by placing 600 μl NMP into each well followed by evacuation of the solution by steam of nitrogen. The washing process is repeated 4 times. The coupling reaction is repeated twice for 1 hour.

Coupling using Mukayama reagent (performed only in certain situations):

Amino acid solution at 650 mM – 40 μl

Mukayama reagent at 111 mg/ml- 60 μl

Collidine added per well- 15 μl

The same procedure as for coupling with PyBroP. Reaction temperature $50^0$C, reaction time: first coupling 4h, second coupling 16h.

Allyl Alloc deprotection: is performed after completing the assembly, by addition of 180 μl solution of 1.5 g $Pd(PPh_3)_4$ in 20 ml $CH_2Cl_2$ containing 5% acetic acid + 2.5% NMM.

Cyclization: this step is performed by addition of 100 μl of PyBoP in NMP + DIEA.

Cleavage of the peptide from the resin and SepPak purification: After final Fmoc deprotection the resin is transferred into a deep well microtiter plate, to each well 300 μl of TFA solution containing 2.5% TIS, 2.5% $H_2O$, 2.5% EDT are added. Removal of the TFA is performed by lyophilization. After cleavage the peptides are purified by SepPak.

Screening of analogs of heat shock proteins for biological activity

Backbone cyclic analogs of hsp60 are screened in-vitro for inhibition of inflammatory reaction mediated by a pro-inflammatory mediator. The method below exemplifies screening for compounds which antagonize the pro-inflammatory action of hsp60, in a similar screening assay that may be used other mediators are applied for induction of a pro-inflammatory response and the backbone cyclized analogs are screened for inhibition of such actions.

Identifying, screening and characterizing compounds which can act as antagonists of hsp60:

The capacity for reduction or preventing the induction of a pro-inflammatory immune response by hsp60 can be determined by using appropriate cells and hsp60 as an inducer. The backbone cyclized analog to be tested is added and the effect on the production of TNFα and/or NO is measured with a decrease or inhibition of TNFα and/or NO production being indicative of the capability of the tested molecule to act as an antagonist of hsp60.

The method comprises the following steps:

a. contacting cells which carry on their surface an intact Toll-like receptor complex with hsp60 or a fragment thereof having the ability to induce a pro-inflammatory response in the presence and in the absence of the compound to be tested; and
b. determining whether the presence of the compound has an effect on the production of TNFα and/or NO of said cells,

wherein a reduction or prevention of the production of TNFα and/or NO is indicative of the ability of the compound to act as an antagonist of hsp60.

The cells used in step (a) of the method are preferably cells carrying an intact Toll-like receptor 4. However, since it is possible that in man the Toll-like receptor 2 is the hsp60 receptor, also cells carrying such a receptor may be used. Preferably, the cells used in the method are lymphatic cells and more preferably macrophages, e.g. human or mouse macrophages. Examples for cells which can be used in such a method are J774 cells, i.e.

the mouse macrophage cell line J774 A.1, which can be purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany) or bone marrow-derived macrophages of mouse strains C57BL/6JBom and C3H/HeN, which can be purchased from Breeding & Research Center A/S (Bomholtgård, Ry, Denmark) and from Charles River (Sulzfeld, Germany), respectively. The preparation of such bone-marrow derived macrophages is well known to the person skilled in the art.

As a negative control bone-marrow-derived cells of C3H/HeJ mice, which can be purchased from Charles River (Sulzfeld, Germany), can be used. These macrophages express a functionally defective Toll-like receptor 4 membrane protein and do not respond to hsp60 by the production of TNFα and NO.

For determining whether a backbone cyclized peptide analog inhibit a pro-inflammatory response induced by hsp60, the cells, i.e. macrophages, are for example seeded in a 96 well flat-bottom microtiter plate ($2x10^5$ cells in 200µl per well). After 24h of preincubation (37°C, 5% $CO_2$) hsp60 is added in addition to the tested compound in various concentrations to the cultures and incubation is continued for different time intervals before measuring the production of TNFα and NO.

Methods for measuring the induction of TNFα and/or NO formation

The induction of TNFα and/or NO formation can be measured in macrophages, e.g., macrophages of C3H/HeN or C57BL/6 mice or J774 cells (Chen et al., ibid), wherein the reduction of TNFα and/or NO formation indicates the ability to inhibit/prevent a pro-inflammatory immune response. The determination of the inhibition of a pro-inflammatory response can, in particular, be carried out as described in Chen et al. (ibid.). Accordingly, the induction of TNFα can be determined by determining the amount of TNFα produced by macrophages before and after exposure to the respective protein, e.g., by using a sandwich ELISA as described in Chen et al., and quantifying TNFα by using a standard curve obtained, e.g., with the recombinant cytokine (Genzyme, Kent, UK) versus medium alone as blank. The induction of NO formation can be determined, e.g., by

measuring the amount of NO released by macrophages by determining the concentration of nitrite ($NO_2^-$) accumulated in the culture supernatant using the colorimetric Griess reaction as described in Chen et al.

Chemiluminescence assay

Phagocytic cells produce, in response to appropriate stimuli, a variety of oxygen-derived toxic radicals all of which are derived from superoxide ($O_2$-). Superoxide is generated by the NADPH-derived one electron reduction of molecular oxygen catalyzed by a membrane-associated heterodimeric flavocytochrome cytb599 composed of gp91phox and p22phox. The conversion of cytb599 to the active state involves dimerization of the cytb599 and the translocation to the membrane and association of four regulatory units.

Proinflammatory agents like LPS modulate NADPH oxidase activity through a priming phenomenon probably involving phosphorylation of at least one of the regulatory proteins. This results in an increased oxidative burst upon stimulation.

Reactive oxygen intermediates are responsible for the primary lesion in diabetes, as the pancreatic ß-cells are exquisitely sensitive to oxidizing radicals. Reactive oxygen intermediates are responsible for the primary lesion in atherosclerosis which is caused by oxidizing lipids in the arterial wall.

The chemiluminescence assay works by detecting the oxygen-derived radicals with luminol which traps the radicals and, as a result, gives off light which is detected in the TopCount scintillation counter.

Cells: J774 cells are plated in white 96 well plates at $2x10^6$ cells/ml, 90µl/well in growth medium including 10% FCS but without phenol red in the RPMI. Cells are allowed to settle at least 2 hours at 37°C.

Luminol: A solution of 10 mg/ml luminol (Sigma A8511) in DMSO is prepared and kept protected from light.

Zymosan: Stock solution of zymosan is prepared by suspending 15 mg of zymosan in 3 ml of saline, boiling for 30 minutes, 2X washing with saline (600g X10 minutes), resuspending in PBS to give a solution of 8.3 mg/ml.

For use: zymosan and luminol are mixed at 1:1 for addition of 10µl/well to cells

Stimulation of cells: Peptides in 10µl are added and incubated overnight at 37°C. A solution of 10µg/ml Hsp60 10µl/well is added.

The plates are incubated 5 hours at 37°C.

Ten-µl solution of Zymosan & luminol is added and plates are incubated at 37°C for 30 minutes. The plate lid is removed and the plate is covered with plastic. Luminescence is read repeatedly using TopCount until the peak of response is reached.

Inhibition of hsp60 binding to Tlr4 may be further or alternatively tested in cell-based or cell-free binding-assays applying a labeled hsp60 and a backbone cyclized peptide in various concentrations competing for binding to the Tlr4. At the end of the incubation period, the amount of labeled (radioactivity for example) hsp60 is determined and the inhibition capacity of the backbone cyclized peptide is calculated.

After the first screening which identify the compounds with potential inhibitory effect on hsp60, the selected compounds are further tested for anti-inflammatory activity and in several animal models for autoimmune diseases. Examples for such assays and models are: influence on insulitis and diabetes in mouse models, as described in WO 96/19236 and WO 97/01959; WO 96/10039 which describes protection against pristane induced arthritis which is an in-vivo model in mice for arthritis; WO 96/16083 which describes an in-vivo method for rheumatoid arthritis model in rat; WO 96/32957 which describes protection against Experimental Allergic Encephalomyelitis (EAE) which is an in-vivo model (in rodents) for multiple sclerosis; and US 5,348,945 which describes an in-vivo model for testing the effect of the compounds on atherosclerosis.

Conformationally constrained analogs of heat shock proteins constructed based in part on the sequences of a number of known heat shock proteins as presented in the examples below. The following examples are intended to illustrate how to make and use the compounds and methods of this invention and are in no way to be construed as a limitation.

## EXAMPLES

The invention will now be illustrated in a non-limitative manner by the following Examples:

**Example 1: Backbone cyclic analogs of p277(Val$^6$, Val$^{11}$) synthesized in multiple parallel synthesis format.**

The peptide designated p277(Val$^6$, Val$^{11}$) is a linear 24-residues-long analog corresponding to residues 436-460 of human hsp60. The peptide, disclosed in US 6,180,103, was identified as a therapeutically useful entity in preventing or alleviating IDDM and host vs. graft disease.

In order to find backbone cyclized analogs of p277(Val$^6$, Val$^{11}$), a set of 96 backbone cyclized peptide analogs is synthesized in MPS format based on its sequence: Val$^1$-Leu$^2$-Gly$^3$-Gly$^4$-Gly$^5$-Val$^6$-Ala$^7$-Leu$^8$-Leu$^9$-Arg$^{10}$-Val$^{11}$-Ile$^{12}$-Pro$^{13}$-Ala$^{14}$-Leu$^{15}$-Asp$^{16}$-Ser$^{17}$-Leu$^{18}$-Thr$^{19}$-Pro$^{20}$-Ala$^{21}$-Asn$^{22}$-Glu$^{23}$-Asp$^{24}$

Wherein underlined residues represent the potential cyclization points.

Cyclization is performed between residue numbers as described in Table No. 1 using the following building units: AlaC2, AlaC3, AlaN2, AlaN3, LeuC3, LeuC4, LeuN3, LeuN4 wherein Ala building unit substitutes Ala residues and Leu building unit substitutes Leu residues of the original peptide sequence.

Table 1. Cyclization points in the sequence of backbone cyclized hsp60 derived peptidomimetics.

| Ala$^7$ | Leu$^8$ | Leu$^9$ | |
|---|---|---|---|
| 7-14 | 8-14 | 9-14 | Ala$^{14}$ |
| 7-15 | 8-15 | 9-15 | Leu$^{15}$ |
| 7-18 | 8-18 | 9-18 | Leu$^{18}$ |
| 7-21 | 8-21 | 9-21 | Ala$^{21}$ |

Numbers indicate the position in the peptide sequence starting from the N-terminus.

**Example 2: Additional backbone cyclic analogs of p277(Val$^6$, Val$^{11}$)**

Additional backbone cyclic analogs are designed which include cyclization between residue Leu$^2$ and additional building unit in the sequence. In additional analogs, the Val residues at positions 6 and 11 are replaced with Cys and bi-cyclic compounds are obtained. Additionally, the free carboxyl of Asp residues at positions 16 and 24 is used to be cyclized with N-type building unit at position 2, 7, 8, 9, 14, 15, 18 to form a backbone-to-side chain cyclization. Any backbone cyclized analog described in examples 1 and 2 may include substituted residue after a building unit in the sequence.

**Example 3: Design of backbone cyclized peptides**

A set of 96 backbone cyclized peptides derived from residues 408-416 of human Hsp60 has been designed. The peptides (Table No. 2) are 9-residues long and comprise four types of cyclizations, bridging between positions 408-414, 409-416, 408-416, and 409-414. Several types of building units (labeled bold in Table No. 2) have been utilized as bridging groups in order to form different bridge sizes and types. The Asn residue at position 415 has been replaced with Gln or another residue due to synthetic considerations.

Table 2.

| A | 408 | 409 | 410 | 411 | 412 | 413 | 414 | 415 | 416 |
|---|---|---|---|---|---|---|---|---|---|
| | **GlyC1** | Thr | Ser | Asp | Val | Glu | **AlaN2** | Gln | Glu |
| | **PheC1** | Thr | Ser | Asp | Val | Glu | **AlaN2** | Gln | Glu |
| | **GlyC2** | Thr | Ser | Asp | Val | Glu | **AlaN2** | Gln | Glu |
| | **TrpC2** | Thr | Ser | Asp | Val | Glu | **AlaN2** | Gln | Glu |
| | **GlyC2** | Thr | Ser | Asp | Val | Glu | **AlaN3** | Gln | Glu |
| | **GlyC3** | Thr | Ser | Asp | Val | Glu | **AlaN2** | Gln | Glu |
| | **GlyC3** | Thr | Ser | Asp | Val | Glu | **AlaN3** | Gln | Glu |
| | **LeuC4** | Thr | Ser | Asp | Val | Glu | **AlaN2** | Gln | Glu |
| | **PheC4** | Thr | Ser | Asp | Val | Glu | **AlaN2** | Gln | Glu |
| | **LysC4** | Thr | Ser | Asp | Val | Glu | **AlaN2** | Gln | Glu |
| | **LeuC4** | Thr | Ser | Asp | Val | Glu | **AlaN3** | Gln | Glu |
| | **PheC4** | Thr | Ser | Asp | Val | Glu | **AlaN3** | Gln | Glu |
| | **LysC4** | Thr | Ser | Asp | Val | Glu | **AlaN3** | Gln | Glu |
| | **LeuC5** | Thr | Ser | Asp | Val | Glu | **AlaN2** | Gln | Glu |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **TrpC5** | Thr | Ser | Asp | Val | Glu | **AlaN3** | Gln | Glu |
| **GlyC2** | Thr | Ser | Asp | Val | Glu | Val | Gln | **LeuN4** |
| **TrpC2** | Thr | Ser | Asp | Val | Glu | Val | Ser | **LeuN4** |
| **GlyN2** | Thr | Ser | Asp | Val | Glu | Val | Gln | **AlaC3** |
| **LeuN2** | Thr | Ser | Asp | Val | Glu | Val | Thr | **PheC3** |
| **SerN2** | Thr | Ser | Asp | Val | Glu | Val | Gln | **AlaC3** |
| **GlyC3** | Thr | Ser | Asp | Val | Glu | Val | Gln | **HISN3** |
| **PheC3** | Thr | Ser | Asp | Val | Glu | Val | Gln | **LysN3** |
| **IleC3** | Thr | Ser | Asp | Val | Glu | Val | Gln | **PheN3** |
| **GlyC3** | Thr | Ser | Asp | Val | Glu | Val | Gln | **PheN3** |
| **GlyC3** | Thr | Ser | Asp | Val | Glu | Val | Ala | **LeuN4** |
| **TrpC3** | Thr | Ser | Asp | Val | Glu | Val | Gln | **GlyN6** |
| **LeuC4** | Thr | Ser | Asp | Val | Glu | Val | Gln | **GluN2** |
| **PheC4** | Thr | Ser | Asp | Val | Glu | Val | Gln | **GluN2** |
| **LysC4** | Thr | Ser | Asp | Val | Glu | Val | Gln | **GluN2** |
| **LeuC4** | Thr | Ser | Asp | Val | Glu | Val | Gln | **LysN2** |
| **PheC4** | Thr | Ser | Asp | Val | Glu | Val | Gln | **PheN2** |
| **GlyN2** | Thr | Ser | Asp | Val | Glu | Val | Gln | **TyrC4** |
| **TrpN2** | Thr | Ser | Asp | Val | Glu | Val | Gln | **AlaC4** |
| **ThrN2** | Thr | Ser | Asp | Val | Glu | Val | Thr | **LeuC4** |
| **SerN2** | Thr | Ser | Asp | Val | Glu | Val | Gln | **MetC4** |
| **LeuN2** | Thr | Ser | Asp | Val | Glu | Val | 2Abu | **PheC4** |
| **LeuC4** | Thr | Ser | Asp | Val | Glu | Val | Gln | **PheN3** |
| **PheC4** | Thr | Ser | Asp | Val | Glu | Val | Gln | **TrpN3** |
| **LysC4** | Thr | Ser | Asp | Val | Glu | Val | Leu | **LeuN3** |
| **LeuC4** | Thr | Ser | Asp | Val | Glu | Val | Thr | **TrpN3** |
| **PheC4** | Thr | Ser | Asp | Val | Glu | Val | Gln | **AlaN3** |
| **LeuC4** | Thr | Ser | Asp | Val | Glu | Val | Gln | **LysN3** |
| **PheC4** | Thr | Ser | Asp | Val | Glu | Val | Gln | **LysN3** |
| **LysC4** | Thr | Ser | Asp | Val | Glu | Val | Gln | **HISN3** |
| **AlaC5** | Thr | Ser | Asp | Val | Glu | Val | Gln | **GluN2** |
| **LeuC5** | Thr | Ser | Asp | Val | Glu | Val | Gln | **GluN2** |
| **TrpC5** | Thr | Ser | Asp | Val | Glu | Val | Gln | **GluN2** |
| **AlaC5** | Thr | Ser | Asp | Val | Glu | Val | Gln | **LysN2** |
| **LeuC5** | Thr | Ser | Asp | Val | Glu | Val | Gln | **AlaN2** |
| **TrpC5** | Thr | Ser | Asp | Val | Glu | Val | Ala | **TrpN2** |
| **LysN2** | Thr | Ser | Asp | Val | Glu | Val | Ser | **LeuC5** |
| **TrpN2** | Thr | Ser | Asp | Val | Glu | Val | Gln | **TrpC5** |
| Gly | **ThrN2** | Ser | Asn | Val | Glu | **AlaC2** | Gln | Glu |
| Gly | **ThrN2** | Ser | Gln | Val | Glu | **AlaC2** | Gln | Glu |
| Gly | **ThrN2** | Ser | Glu | Val | Glu | **AlaC2** | Gln | Glu |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Gly | **ThrN2** | Ser | Lys | Val | Glu | **AlaC2** | Gln | Glu |
| Gly | **ThrN2** | Ser | Tyr | Val | Glu | **AlaC2** | Gln | Lys |
| Gly | **SerN2** | Ser | Asn | Val | Tyr | **AlaC2** | Gln | Glu |
| Gly | **SerN2** | Ser | Gln | Val | Glu | **AlaC2** | Gln | Glu |
| Gly | **ThrN2** | 2Abu | Asn | Val | Glu | **AlaC2** | Gln | Glu |
| Gly | **ThrN2** | Ser | Gln | Val | Glu | **AlaC2** | Gln | Asp |
| Gly | **ThrN2** | Ser | Glu | Val | Glu | **AlaC2** | Gln | Tyr |
| Gly | **ThrN2** | Ser | Lys | Val | Glu | **AlaC2** | Gln | Phe |
| Gly | **AlaC3** | Ser | Asp | Val | Glu | **AlaN2** | Gln | Glu |
| Gly | **LeuC3** | Ser | Asp | Val | Glu | **AlaN2** | Gln | Glu |
| Gly | **PheC3** | Ser | Asp | Val | Glu | **AlaN2** | Gln | Glu |
| Gly | **AlaC3** | Ser | Asp | Val | Glu | **AlaN3** | Gln | Glu |
| Gly | **LeuC3** | Ser | Asp | Val | Glu | **AlaN3** | Gln | Glu |
| Gly | **PheC3** | Ser | Asp | Val | Glu | **AlaN3** | Gln | Glu |
| Gly | **ThrN2** | Ser | Asp | Val | Glu | **AlaC2** | Gln | Glu |
| Gly | **ThrN2** | Thr | Asp | Val | Glu | **AlaC2** | Gln | Asp |
| Gly | **ThrN2** | Val | Asp | Leu | Glu | **AlaC2** | Gln | Lys |
| Gly | **ThrN2** | 2Abu | Asp | 2Abu | Glu | **AlaC2** | Gln | Glu |
| Gly | **ThrN2** | Phe | Asp | Ile | Glu | **AlaC2** | Gln | Glu |
| Gly | **SerN2** | Leu | Asp | Ala | Glu | **AlaC2** | Gln | Glu |
| Gly | **SerN2** | Thr | Asp | Leu | Glu | **AlaC2** | Gln | Glu |
| Gly | **ThrN2** | Ser | Asp | Val | Glu | Val | Gln | **TyrC4** |
| Gly | **ThrN2** | Ser | Asp | Val | Glu | Val | Tyr | **LeuC4** |
| Gly | **LeuC3** | Ser | Asp | Val | Glu | Val | Gln | **GluN2** |
| Gly | **PheC3** | Ser | Asp | Val | Glu | Val | Gln | **GluN2** |
| Gly | **ValC3** | Ser | Asp | Val | Glu | Val | Gln | **GluN2** |
| Gly | **LeuC3** | Ser | Asp | Val | Glu | Val | Gln | **PheN3** |
| Gly | **PheC3** | Ser | Asp | Val | Glu | Val | Thr | **TyrN3** |
| Gly | **ValC3** | 2Abu | Asp | Val | Glu | Val | Gln | **PheN3** |
| Gly | **LeuC3** | Ser | Asp | Val | Glu | Val | Gln | **TyrN3** |
| Gly | **PheC3** | Ser | Asp | Val | Glu | Val | Gln | **TyrN3** |
| Gly | **ValC3** | Ser | Asp | Val | Glu | Val | Gln | **PheN3** |
| Gly | **LeuC3** | Ser | Asp | Val | Glu | Val | 2Abu | **TyrN3** |
| Gly | **PheC3** | Ser | Asp | Val | Glu | Val | Gln | **PheN3** |
| Gly | **ValC3** | Ser | Asp | Val | Glu | Val | Gln | **TyrN3** |
| Gly | Thr | Ser | Asp | Val | Glu | Val | Gln | Glu |

Last peptide is the native sequence

**Example 4: Manufacture of a medicament containing synthetic peptide analogs of the invention**

As used herein a "pharmaceutical composition" refers to a preparation of one or more of the peptides described herein, or physiologically acceptable salts or prodrugs thereof, with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of a compound. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Pharmaceutical compositions may also include one or more additional active ingredients, such as, but not limited to, conventional anti-inflammatory agents.

Pharmaceutical compositions of the present invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, grinding, pulverizing, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active compounds into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the compounds of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants for example DMSO, or polyethylene glycol are generally known in the art.

The term "prodrug" refers to an agent, which is converted into an active parent drug *in vivo*. Prodrugs are often useful because in some instances they may be easier to

administer than the parent drug. They may, for instance, be bioavailable by oral administration whereas the parent drug is not. The prodrug may also have improved solubility compared to the parent drug in pharmaceutical compositions.

While it is known that peptides are not normally orally available, use of the analogs of the present invention may enhance oral bioavailability.

For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carbomethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. In addition enterocoating are useful as it is desirable to prevent exposure of the peptides of the invention to the gastric environment.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions, which can be used orally, include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture

with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for the chosen route of administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the peptides for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichloro-tetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the peptide and a suitable powder base such as lactose or starch.

Pharmaceutical compositions for parenteral administration include aqueous solutions of the active ingredients in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents, which increase the solubility of the compounds, to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for reconstitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.

The compounds of the present invention may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

The pharmaceutical compositions herein described may also comprise suitable solid of gel phase carriers or excipients. Examples of such carriers or excipients include, but are not limited to, calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin and polymers such as polyethylene glycols.

Pharmaceutical compositions suitable for use in context of the present invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. More specifically, a therapeutically effective amount means an amount of a compound effective to prevent, alleviate or ameliorate symptoms of a disease of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

Toxicity and therapeutic efficacy of the peptides described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., by determining the $IC_{50}$ (the concentration which provides 50% inhibition) and the $LD_{50}$ (lethal dose causing death in 50 % of the tested animals) for a subject compound. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

Depending on the severity and responsiveness of the condition to be treated, dosing can also be a single administration of a slow release composition, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, and all other relevant factors.

The following example is an illustration only of a method of treating a subject with a peptide according to the invention, in order to treat a pathological condition associated with tissue trauma or a related condition, and is not intended to be limiting.

The method includes the step of administering the protective peptide, in a pharmaceutically acceptable carrier as described above, to a subject to be treated. The medicament is administered according to an effective dosing methodology, preferably until a predefined endpoint is reached, such as a reduction or amelioration of the pathological condition in the subject.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims. All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention.

## THE CLAIMS

What is claimed is:

1. A backbone cyclized analog of a heat shock protein or a fragment thereof comprising a peptide sequence of five to thirty amino acids that incorporates at least one building unit, said building unit containing one nitrogen atom of the peptide backbone connected to a bridging group comprising an amide, thioether, thioester or disulfide, wherein the at least one building unit is connected via the bridging group to form a cyclic structure with a moiety selected from the group consisting of a second building unit, the side chain of an amino acid residue of the sequence or a terminal amino acid residue.

2. A backbone cyclized antagonist of a heat shock protein or a fragment thereof comprising a peptide sequence of five to thirty amino acids that incorporates at least one building unit, said building unit containing one nitrogen atom of the peptide backbone connected to a bridging group comprising an amide, thioether, thioester or disulfide, wherein the at least one building unit is connected via the bridging group to form a cyclic structure with a moiety selected from the group consisting of a second building unit, the side chain of an amino acid residue of the sequence or a terminal amino acid residue.

3. The backbone cyclized analog of claim 1 or 2 wherein the peptide sequence is derived from hsp60.

4. The backbone cyclized analog according to claim 1 or 2 capable of binding to the toll-like-receptor-4.

5. The backbone cyclized analog according to claim 1 or 2 which is an analog of a sequence selected from the group of:

a. Glu-Glu-Ile-Ala-Gln-Val-Ala-Thr-Ile-Ser-Ala-Asn-Gly-Asp-Lys-Glu-Ile-Gly-Asn-Ile corresponding to residues 166-185 of human hsp60;

b. Val-Leu-Gly-Gly-Gly-Cys-Ala-Leu-Leu-Arg-X2-Ile-Pro-Ala-Leu-Asp-Ser-Leu-Cys-Pro-Ala-Asn-Glu-Asp corresponding to residues 437-460 of the human hsp60, wherein Cys at positions 442 and 447 may be each substituted with Val residue and Thr at position 450 may be substituted with Lys;

c. Ile-Val-Leu-Gly-Gly-Gly-Cys-Ala-Leu-Leu-Arg-Cys-Ile-Pro-Ala-Leu-Asp-Ser-Leu-Thr corresponding to residues 436-455 of human hsp60;

d. Glu-Ile-Ile-Lys-Arg-Thr-Leu-Lys-Ile-Pro-Ala-Met-Thr-Ile-Ala-Lys-Asn-Ala-Gly-Val corresponding to residues 466-485 of human hsp60;

e. Thr-Phe-Gly-Leu-Gln-Leu-Glu-Leu-Thr-Glu-Gly-Met-Arg-Phe-Asp-Lys corresponding to residues 135-150 of Mycobacterium avium hsp65;

f. Gly-Val-Ile-Thr-Val-Glu-Glu-Ser-Asn-Thr-Phe-Gly-Leu-Gln-Leu-Glu-Leu-Thr-Glu-Gly-Met-Arg-Phe-Asp-Lys-Gly-Tyr-Ile-Ser-Gly-Tyr-Phe-Val-Thr-Asp corresponding to residues 171-240 of the bacterial Antigen A;

g. Pro-Glu-Arg-Gln-Glu-Ala-Val-Leu-Glu-Asp-Pro-Tyr-Ile-Leu-Leu-Val-Ser-Ser-Lys-Val-Ser-Thr-Val-Lys-Asp-Leu-Leu-Pro-Leu-Leu-Glu-Lys-Val-Ile-Gly corresponding to residues 207-241 of the bacterial Antigen A;

h. Thr-Phe-Gly-Leu-Gln-Leu-Glu corresponding to residues 180-186 of Mycobacterium tuberculosis hsp65 and residues 135-141 of Mycobacterium avium hsp65, wherein any of residues Phe, Gly and Leu may be optionally substituted with another residue; Thr-AA1-AA2-AA3-Gln-Leu-Glu wherein AA1, AA2 and AA3 designate any amino acid residue;

i. Ile-Val-Gly-Leu-Thr-Leu-Glu-Asn-Ala-Asp-Leu-Ser-Leu derived from the sequence of microbial hsp60;

j. Val-Leu-Asn-Arg-Leu-Lys-Val-Gly-Leu-Gln-Val derived from the sequence of human hsp60;

k. Leu-Thr-Leu-Asn-Leu-Glu-Asp-Val-Gln-Pro-His-Asp corresponding to residues 330 to 341 of human hsp60;

l. Ala-Lys-Val-Asn-Ile-Lys-Pro-Leu-Glu-Asp-Lys-Ile-Leu-Val-Gln-Ala-Asn-Glu-Ala-Glu-Thr-Thr-Thr corresponding to residues 2 to 26 of Mycobacterium hsp10;

m. Thr-Ile-Ala-Ser-Asp-Glu-Glu-Ala-Arg-Arg-Gly-Leu corresponding to residues 3 to 14 of Mycobacterium hsp60;

n. Thr-Ile-Ala-Ser-Asp-Glu-Glu corresponding to residues 3-9 of Mycobacterium hsp60, wherein the residue Ser (at position 6) may be optionally substituted with Arg or Pro;

o. Thr-Ile-His-Tyr-Asp-Glu-Glu corresponding to residues 3-9 of Mycobacterium hsp60, wherein the residue His (at position 5) may be optionally substituted with Gln;

p. Gly-Pro-Lys-Gly-Arg-Asn-Val-Val-Leu-Glu-Lys-Lys-Trp-Gly-Ala-Pro corresponding to residues 31 to 46 of Mycobacterium tuberculosis hsp60;

q. Val-Val-Leu-Glu-Lys-Lys-Trp-Gly-Ala-Pro-Thr-Ile-Thr-Asn-Asp-Gly corresponding to residues 37 to 52 of Mycobacterium tuberculosis hsp60;

r. Thr-Val-Ile-Ile-Glu-Gln-Ser-Trp-Gly-Ser-Pro-Lys-Val-Thr-Lys-Asp-Gly-Val-Thr-Val corresponding to residues 36 to 55 of Mycobacterium tuberculosis hsp60;

s. Val-Val-Asn-Lys-Ile-Arg-Gly corresponding to residues 261-271of bacterial hsp65;

t. Leu-Lys-Pro-Gly-Leu-Glu-Lys-Asp-Phe derived from the sequence of Mycobacterial hsp60;

u. Leu-Lys-Arg-Gly-Ile-Glu-Lys-Ala-Val corresponding to residues derived from the sequence of Mycobacterial hsp60;

v. Val-Ala-Vla-Lys-Ala-Pro-Gly-Phe-Gly-Asp-Arg-Arg-Lys-Ala-Met corresponding to residues 272-286 of Mycobacterial lepae.

6. A pharmaceutical composition comprising a backbone cyclized inhibitor of heat shock protein comprising a peptide sequence of five to thirty amino acids that incorporates at least one building unit, said building unit containing one nitrogen atom of the peptide backbone connected to a bridging group comprising an amide, thioether,

thioester or disulfide, wherein the at least one building unit is connected via the bridging group to form a cyclic structure with a moiety selected from the group consisting of a second building unit, the side chain of an amino acid residue of the sequence or a terminal amino acid residue, and a pharmaceutically acceptable carrier.

7. The pharmaceutical composition of claim 6 comprising as an active ingredient a backbone cyclized analog of a heat shock protein wherein the peptide sequence is derived from hsp60.

8. The pharmaceutical composition of claim 7 wherein the analog is an analog of a sequence selected from the group of:

a. Glu-Glu-Ile-Ala-Gln-Val-Ala-Thr-Ile-Ser-Ala-Asn-Gly-Asp-Lys-Glu-Ile-Gly-Asn-Ile corresponding to residues 166-185 of human hsp60;

b. Val-Leu-Gly-Gly-Gly-Cys-Ala-Leu-Leu-Arg-X2-Ile-Pro-Ala-Leu-Asp-Ser-Leu-Cys-Pro-Ala-Asn-Glu-Asp corresponding to residues 437-460 of the human hsp60, wherein Cys at positions 442 and 447 may be each substituted with Val residue and Thr at position 450 may be substituted with Lys;

c. Ile-Val-Leu-Gly-Gly-Gly-Cys-Ala-Leu-Leu-Arg-Cys-Ile-Pro-Ala-Leu-Asp-Ser-Leu-Thr corresponding to residues 436-455 of human hsp60;

d. Glu-Ile-Ile-Lys-Arg-Thr-Leu-Lys-Ile-Pro-Ala-Met-Thr-Ile-Ala-Lys-Asn-Ala-Gly-Val corresponding to residues 466-485 of human hsp60;

e. Thr-Phe-Gly-Leu-Gln-Leu-Glu-Leu-Thr-Glu-Gly-Met-Arg-Phe-Asp-Lys corresponding to residues 135-150 of Mycobacterium avium hsp65;

f. Gly-Val-Ile-Thr-Val-Glu-Glu-Ser-Asn-Thr-Phe-Gly-Leu-Gln-Leu-Glu-Leu-Thr-Glu-Gly-Met-Arg-Phe-Asp-Lys-Gly-Tyr-Ile-Ser-Gly-Tyr-Phe-Val-Thr-Asp corresponding to residues 171-240 of the bacterial Antigen A;

g. Pro-Glu-Arg-Gln-Glu-Ala-Val-Leu-Glu-Asp-Pro-Tyr-Ile-Leu-Leu-Val-Ser-Ser-Lys-Val-Ser-Thr-Val-Lys-Asp-Leu-Leu-Pro-Leu-Leu-Glu-Lys-Val-Ile-Gly corresponding to residues 207-241 of the bacterial Antigen A;

h. Thr-Phe-Gly-Leu-Gln-Leu-Glu corresponding to residues 180-186 of Mycobacterium tuberculosis hsp65 and residues 135-141 of Mycobacterium avium hsp65, wherein any of residues Phe, Gly and Leu may be optionally substituted with another residue; Thr-AA1-AA2-AA3-Gln-Leu-Glu wherein AA1, AA2 and AA3 designate any amino acid residue;

i. Ile-Val-Gly-Leu-Thr-Leu-Glu-Asn-Ala-Asp-Leu-Ser-Leu derived from the sequence of microbial hsp60;

j. Val-Leu-Asn-Arg-Leu-Lys-Val-Gly-Leu-Gln-Val derived from the sequence of human hsp60;

k. Leu-Thr-Leu-Asn-Leu-Glu-Asp-Val-Gln-Pro-His-Asp corresponding to residues 330 to 341 of human hsp60;

l. Ala-Lys-Val-Asn-Ile-Lys-Pro-Leu-Glu-Asp-Lys-Ile-Leu-Val-Gln-Ala-Asn-Glu-Ala-Glu-Thr-Thr-Thr corresponding to residues 2 to 26 of Mycobacterium hsp10;

m. Thr-Ile-Ala-Ser-Asp-Glu-Glu-Ala-Arg-Arg-Gly-Leu corresponding to residues 3 to 14 of Mycobacterium hsp60;

n. Thr-Ile-Ala-Ser-Asp-Glu-Glu corresponding to residues 3-9 of Mycobacterium hsp60, wherein the residue Ser (at position 6) may be optionally substituted with Arg or Pro;

o. Thr-Ile-His-Tyr-Asp-Glu-Glu corresponding to residues 3-9 of Mycobacterium hsp60, wherein the residue His (at position 5) may be optionally substituted with Gln;

p. Gly-Pro-Lys-Gly-Arg-Asn-Val-Val-Leu-Glu-Lys-Lys-Trp-Gly-Ala-Pro corresponding to residues 31 to 46 of Mycobacterium tuberculosis hsp60;

q. Val-Val-Leu-Glu-Lys-Lys-Trp-Gly-Ala-Pro-Thr-Ile-Thr-Asn-Asp-Gly corresponding to residues 37 to 52 of Mycobacterium tuberculosis hsp60;

r. Thr-Val-Ile-Ile-Glu-Gln-Ser-Trp-Gly-Ser-Pro-Lys-Val-Thr-Lys-Asp-Gly-Val-Thr-Val corresponding to residues 36 to 55 of Mycobacterium tuberculosis hsp60;

s. Val-Val-Asn-Lys-Ile-Arg-Gly corresponding to residues 261-271of bacterial hsp65;

t. Leu-Lys-Pro-Gly-Leu-Glu-Lys-Asp-Phe derived from the sequence of Mycobacterial hsp60;

u. Leu-Lys-Arg-Gly-Ile-Glu-Lys-Ala-Val corresponding to residues derived from the sequence of Mycobacterial hsp60;

v. Val-Ala-Vla-Lys-Ala-Pro-Gly-Phe-Gly-Asp-Arg-Arg-Lys-Ala-Met corresponding to residues 272-286 of Mycobacterial lepae.

9. A method for treating disorders selected from the group consisting of chronic inflammatory diseases, autoimmune diseases, infectious diseased and graft rejection comprising administering to a patient in need thereof a pharmaceutical composition comprising a therapeutically effective amount of a backbone cyclized analog of heat shock protein.

10. The method of claim 9 comprising a therapeutically effective amount of a backbone cyclized analog of a heat shock protein, wherein the peptide sequence is derived from hsp60.

11. The method of claim 10 wherein the analog is an analog of a sequence selected from the group of:

a. Glu-Glu-Ile-Ala-Gln-Val-Ala-Thr-Ile-Ser-Ala-Asn-Gly-Asp-Lys-Glu-Ile-Gly-Asn-Ile corresponding to residues 166-185 of human hsp60;

b. Val-Leu-Gly-Gly-Gly-Cys-Ala-Leu-Leu-Arg-X2-Ile-Pro-Ala-Leu-Asp-Ser-Leu-Cys -Pro-Ala-Asn-Glu-Asp corresponding to residues 437-460 of the human hsp60, wherein Cys at positions 442 and 447 may be each substituted with Val residue and Thr at position 450 may be substituted with Lys;

c. Ile-Val-Leu-Gly-Gly-Gly-Cys-Ala-Leu-Leu-Arg-Cys-Ile-Pro-Ala-Leu-Asp-Ser-Leu -Thr corresponding to residues 436-455 of human hsp60;

d. Glu-Ile-Ile-Lys-Arg-Thr-Leu-Lys-Ile-Pro-Ala-Met-Thr-Ile-Ala-Lys-Asn-Ala-Gly-Val corresponding to residues 466-485 of human hsp60;

e. Thr-Phe-Gly-Leu-Gln-Leu-Glu-Leu-Thr-Glu-Gly-Met-Arg-Phe-Asp-Lys corresponding to residues 135-150 of Mycobacterium avium hsp65;

f. Gly-Val-Ile-Thr-Val-Glu-Glu-Ser-Asn-Thr-Phe-Gly-Leu-Gln-Leu-Glu-Leu-Thr-Glu -Gly-Met-Arg-Phe-Asp-Lys-Gly-Tyr-Ile-Ser-Gly-Tyr-Phe-Val-Thr-Asp corresponding to residues 171-240 of the bacterial Antigen A;

g. Pro-Glu-Arg-Gln-Glu-Ala-Val-Leu-Glu-Asp-Pro-Tyr-Ile-Leu-Leu-Val-Ser-Ser-Lys -Val-Ser-Thr-Val-Lys-Asp-Leu-Leu-Pro-Leu-Leu-Glu-Lys-Val-Ile-Gly corresponding to residues 207-241 of the bacterial Antigen A;

h. Thr-Phe-Gly-Leu-Gln-Leu-Glu corresponding to residues 180-186 of Mycobacterium tuberculosis hsp65 and residues 135-141 of Mycobacterium avium hsp65, wherein any of residues Phe, Gly and Leu may be optionally substituted with another residue; Thr-AA1-AA2-AA3-Gln-Leu-Glu wherein AA1, AA2 and AA3 designate any amino acid residue;

i. Ile-Val-Gly-Leu-Thr-Leu-Glu-Asn-Ala-Asp-Leu-Ser-Leu derived from the sequence of microbial hsp60;

j. Val-Leu-Asn-Arg-Leu-Lys-Val-Gly-Leu-Gln-Val derived from the sequence of human hsp60;

k. Leu-Thr-Leu-Asn-Leu-Glu-Asp-Val-Gln-Pro-His-Asp corresponding to residues 330 to 341 of human hsp60;

l. Ala-Lys-Val-Asn-Ile-Lys-Pro-Leu-Glu-Asp-Lys-Ile-Leu-Val-Gln-Ala-Asn-Glu-Ala -Glu-Thr-Thr-Thr corresponding to residues 2 to 26 of Mycobacterium hsp10;

m. Thr-Ile-Ala-Ser-Asp-Glu-Glu-Ala-Arg-Arg-Gly-Leu corresponding to residues 3 to 14 of Mycobacterium hsp60;

n. Thr-Ile-Ala-Ser-Asp-Glu-Glu corresponding to residues 3-9 of Mycobacterium hsp60, wherein the residue Ser (at position 6) may be optionally substituted with Arg or Pro;

o. Thr-Ile-His-Tyr-Asp-Glu-Glu corresponding to residues 3-9 of Mycobacterium hsp60, wherein the residue His (at position 5) may be optionally substituted with Gln;

p. Gly-Pro-Lys-Gly-Arg-Asn-Val-Val-Leu-Glu-Lys-Lys-Trp-Gly-Ala-Pro corresponding to residues 31 to 46 of Mycobacterium tuberculosis hsp60;

q. Val-Val-Leu-Glu-Lys-Lys-Trp-Gly-Ala-Pro-Thr-Ile-Thr-Asn-Asp-Gly

corresponding to residues 37 to 52 of Mycobacterium tuberculosis hsp60;

r. Thr-Val-Ile-Ile-Glu-Gln-Ser-Trp-Gly-Ser-Pro-Lys-Val-Thr-Lys-Asp-Gly-Val-Thr-Val corresponding to residues 36 to 55 of Mycobacterium tuberculosis hsp60;

s. Val-Val-Asn-Lys-Ile-Arg-Gly corresponding to residues 261-271of bacterial hsp65;

t. Leu-Lys-Pro-Gly-Leu-Glu-Lys-Asp-Phe derived from the sequence of Mycobacterial hsp60;

u. Leu-Lys-Arg-Gly-Ile-Glu-Lys-Ala-Val corresponding to residues derived from the sequence of Mycobacterial hsp60;

v. Val-Ala-Vla-Lys-Ala-Pro-Gly-Phe-Gly-Asp-Arg-Arg-Lys-Ala-Met corresponding to residues 272-286 of Mycobacterial lepae.

12. A method for diagnosing autoimmune and inflammatory diseases including a backbone cyclized heat shock protein analog comprising a peptide sequence of five to thirty amino acids that incorporates at least one building unit, said building unit containing one nitrogen atom of the peptide backbone connected to a bridging group comprising an amide, thioether, thioester or disulfide, wherein the at least one building unit is connected via the bridging group to form a cyclic structure with a moiety selected from the group consisting of a second building unit, the side chain of an amino acid residue of the sequence or a terminal amino acid residue.

13. The method for claim 12 comprising a backbone cyclized analog of heat shock protein, wherein the peptide sequence is derived from hsp60.

14. The method for claim 13 comprising a backbone cyclized analog of heat shock protein, wherein the analog is an analog of a sequence selected from the group of:

a. Glu-Glu-Ile-Ala-Gln-Val-Ala-Thr-Ile-Ser-Ala-Asn-Gly-Asp-Lys-Glu-Ile-Gly-Asn-Ile corresponding to residues 166-185 of human hsp60;

b. Val-Leu-Gly-Gly-Gly-Cys-Ala-Leu-Leu-Arg-X2-Ile-Pro-Ala-Leu-Asp-Ser-Leu-Cys-Pro-Ala-Asn-Glu-Asp corresponding to residues 437-460 of the human hsp60,

wherein Cys at positions 442 and 447 may be each substituted with Val residue and Thr at position 450 may be substituted with Lys;

c. Ile-Val-Leu-Gly-Gly-Gly-Cys-Ala-Leu-Leu-Arg-Cys-Ile-Pro-Ala-Leu-Asp-Ser-Leu -Thr corresponding to residues 436-455 of human hsp60;

d. Glu-Ile-Ile-Lys-Arg-Thr-Leu-Lys-Ile-Pro-Ala-Met-Thr-Ile-Ala-Lys-Asn-Ala-Gly-Val corresponding to residues 466-485 of human hsp60;

e. Thr-Phe-Gly-Leu-Gln-Leu-Glu-Leu-Thr-Glu-Gly-Met-Arg-Phe-Asp-Lys corresponding to residues 135-150 of Mycobacterium avium hsp65;

f. Gly-Val-Ile-Thr-Val-Glu-Glu-Ser-Asn-Thr-Phe-Gly-Leu-Gln-Leu-Glu-Leu-Thr-Glu -Gly-Met-Arg-Phe-Asp-Lys-Gly-Tyr-Ile-Ser-Gly-Tyr-Phe-Val-Thr-Asp corresponding to residues 171-240 of the bacterial Antigen A;

g. Pro-Glu-Arg-Gln-Glu-Ala-Val-Leu-Glu-Asp-Pro-Tyr-Ile-Leu-Leu-Val-Ser-Ser-Lys -Val-Ser-Thr-Val-Lys-Asp-Leu-Leu-Pro-Leu-Leu-Glu-Lys-Val-Ile-Gly corresponding to residues 207-241 of the bacterial Antigen A;

h. Thr-Phe-Gly-Leu-Gln-Leu-Glu corresponding to residues 180-186 of Mycobacterium tuberculosis hsp65 and residues 135-141 of Mycobacterium avium hsp65, wherein any of residues Phe, Gly and Leu may be optionally substituted with another residue; Thr-AA1-AA2-AA3-Gln-Leu-Glu wherein AA1, AA2 and AA3 designate any amino acid residue;

i. Ile-Val-Gly-Leu-Thr-Leu-Glu-Asn-Ala-Asp-Leu-Ser-Leu derived from the sequence of microbial hsp60;

j. Val-Leu-Asn-Arg-Leu-Lys-Val-Gly-Leu-Gln-Val derived from the sequence of human hsp60;

k. Leu-Thr-Leu-Asn-Leu-Glu-Asp-Val-Gln-Pro-His-Asp corresponding to residues 330 to 341 of human hsp60;

l. Ala-Lys-Val-Asn-Ile-Lys-Pro-Leu-Glu-Asp-Lys-Ile-Leu-Val-Gln-Ala-Asn-Glu-Ala -Glu-Thr-Thr-Thr corresponding to residues 2 to 26 of Mycobacterium hsp10;

m. Thr-Ile-Ala-Ser-Asp-Glu-Glu-Ala-Arg-Arg-Gly-Leu corresponding to residues 3 to 14 of Mycobacterium hsp60;

n. Thr-Ile-Ala-Ser-Asp-Glu-Glu corresponding to residues 3-9 of Mycobacterium hsp60, wherein the residue Ser (at position 6) may be optionally substituted with Arg or Pro;

o. Thr-Ile-His-Tyr-Asp-Glu-Glu corresponding to residues 3-9 of Mycobacterium hsp60, wherein the residue His (at position 5) may be optionally substituted with Gln;

p. Gly-Pro-Lys-Gly-Arg-Asn-Val-Val-Leu-Glu-Lys-Lys-Trp-Gly-Ala-Pro corresponding to residues 31 to 46 of Mycobacterium tuberculosis hsp60;

q. Val-Val-Leu-Glu-Lys-Lys-Trp-Gly-Ala-Pro-Thr-Ile-Thr-Asn-Asp-Gly corresponding to residues 37 to 52 of Mycobacterium tuberculosis hsp60;

r. Thr-Val-Ile-Ile-Glu-Gln-Ser-Trp-Gly-Ser-Pro-Lys-Val-Thr-Lys-Asp-Gly-Val-Thr-Val corresponding to residues 36 to 55 of Mycobacterium tuberculosis hsp60;

s. Val-Val-Asn-Lys-Ile-Arg-Gly corresponding to residues 261-271of bacterial hsp65;

t. Leu-Lys-Pro-Gly-Leu-Glu-Lys-Asp-Phe derived from the sequence of Mycobacterial hsp60;

u. Leu-Lys-Arg-Gly-Ile-Glu-Lys-Ala-Val corresponding to residues derived from the sequence of Mycobacterial hsp60;

v. Val-Ala-Vla-Lys-Ala-Pro-Gly-Phe-Gly-Asp-Arg-Arg-Lys-Ala-Met corresponding to residues 272-286 of Mycobacterial lepae.